REMARKS/ARGUMENTS

Reconsideration of the Rejections and Objections of the Office Action and allowance of the claims are respectfully requested in view of Applicants' remarks below.

Remarks Regarding Amendments

Support for the New Title and New Abstract may be found in pending claim 1. Applicants have amended claims 1, 2, 3, 5, 7, 8 and 25 to state even more clearly that the method relates to determining a synergetic effect of a multicomponent natural product mixture. Support for this amendment may be found throughout the Specification such as, for example, in claim 1 (see, e.g., preamble) and in paragraphs [0028] and [0032] of the published Specification.

Remarks Regarding IDS

The Office Action objected to the IDS filed October 22, 2010. To expedite prosecution, Applicants have submitted an IDS on March 10, 2011 to supplement the IDS of October 22, 2010.

Related Applications

The Examiner requested information on all related application and their status. Applicants' response is as follows: Applicants direct the Examiner to related and pending Application 10/571,087. A non-Final Rejection has been issued in this Application on January 12, 2011.

Elected Species

Applicants confirm that the elected species is biomarkers and each of the pending claims, claims 1-20 and 25, encompasses the elected species.

Remarks Regarding Section 103

A claimed invention is unpatentable if the differences between it and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art. *In re Kahn*, 78 USPQ2d 1329, 1334 (Fed. Cir. 2006) citing *Graham v. John Deere*, 148 USPQ 459 (1966). The *Graham* analysis needs to be made explicitly. *KSR v. Teleflex*, 82 USPQ2d 1385, 1396 (2007). It requires findings of fact and a rational basis for combining the prior art disclo-sures to produce the claimed invention. See id. ("Often, it will be necessary for a court to look to interrelated teachings of multiple patents . . . and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue"). The use of hindsight reasoning is impermissible. See id. at 1397 ("A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon ex post reasoning"). Thus, a prima facie case under Section 103(a) requires "some rationale, articulation, or reasoned basis to explain why the conclusion of obviousness is correct." *Kahn* at 1335; see *KSR* at 1396.

Claims 1-20, and 25 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over each of Huyn (U.S. Publication No. 2002/0095260), Borisy (U.S. Publication

No. 2003/0096309), Afeyan (U.S. Publication No. 2005/0283320), Khwaja (U.S. Patent No. 6,379,714) or Pugh (J. Argicultural Food Chem. entitled "Characterization of Aloeride, A new High MW Polysaccharide form Aloe vera with Potent Immunostimulatory Activity"). Applicants traverse.

The Features of the Claims

The present application provides a method to identify, within a complex multicomponent natural product, the components or groups of components and their respective concentrations which are required for producing a **synergistic effect** on the biological profile of a disease. The identification of these components provides a scientific basis for testing the efficacy and safety of complex natural products (see, e.g., page 7, lines 10-12 of Applicants' Specification). The present invention, in contrast to the prior art, relates to the characterization of multiple components within a complex mixture using a multivariate analysis. Therefore, both the data input (product mixture) and the information extracted (biological profile) from the analysis is multifactorial in nature.

Regarding the Specific Points Discussed by the Examiner in the Section 103 Rejection

In particular, the Examiner argues that our previous arguments are not enabled because the claims do not cover the subject matter of the claimed invention. Specifically, the Examiner argues that (1) there is no synergy in the Specification and the claims, (2) no feedback signaling

is claimed; (3) the claims are not directed to synergism and (4) employing multiple markers are not claimed.

With respect to the Examiner's points (1) and (3), Applicants note that the claimed method relates to detection of synergistic effects. This can be seen in the preamble to claim 1. Since claim 1 is the sole independent claim, this feature is also incorporated by reference into every other pending claim. In addition, solely in an effort to expedite prosecution, the steps of the claims 1, 2, 3, 5, 7, 8 and 25 have been amended to recite even more clearly that the claimed method involves synergistic effects. This detection of synergistic effect may be found in published paragraphs [0028] and [0032] of the Specification as follows:

> **[0028]** The determination of biological effects and in particular synergetic multicomponent effects in accordance with the present invention is illustrated by the example of herbal medicine products in intervention strategies. The determination of such effects is not limited to biological and synergetic effects in mammalian systems but can address all possible forms of living systems with complex mixtures derived from the same portfolio of life.
>
> **[0032]** A further very important aspect of the method according to the present invention is that it allows the measurement of all biological effects including additive and synergetic effects.

With respect to point (4), the independent claim 1 has been amended to recite that multiple markers are used ("the biological profiles are determined using more than one of the following biomarkers; genes, transcripts, proteins, metabolites and trace elements"). With respect to point (2), Applicants note that claim 1 determines a synergistic effect by determining a profile of a disease in step (b), then the synergistic effect of a multicomponent mixture is

determined in step (c), based on this feedback, identifying within the compositions as determined in step (c) the effective natural components or groups of natural components and their respective concentrations required for having said synergetic effect on the biological profile of the disease, using a multivariate analysis is performed in step (d). Therefore, steps (b), (c) and (d) refer to feedback signaling.

Regarding the Current Rejection

For the reasons stated above, and in view of Applicants amendment further clarifying the features of the claimed invention, Applicants argument for non-obviousness of the claims over each of the cited references remains valid. Specifically, none of the cited art documents relate to methods for determining components of a natural product having a synergistic effect on the complex biological profile of a disease. Borisy *et al.* describe a method of target-centric screening for drug-drug interactions using a library of synthetic or purified compounds. Huyn describes a biological marker identification method that can be used to assess response to a drug. Afeyan *et al.* relates to a method for profiling a biological system. None of these references relate to multicomponent natural products or to the problem of identifying components within said products that produce a synergistic effect.

Pugh *et al.* describe the effects of one component (Aloeride) of aloe gel in *in vitro* assays. Khwaja *et al.* describe methods for making pharmaceutical grade botanical materials, whereby the biological activity of the botanical material as a whole is assayed. These further references

are also unrelated to the problem of identifying components within multicomponent natural products that produce a synergistic effect.

The Examiner states on page 4 of the Office Action that "most all drugs originated in natural products . . . and the desired activity was found in some fashion to be associated with a specific and **single** chemical . . ." (emphasis added). By contrast, the present invention is directed to identifying **more than one** components of natural products that act **synergistically**.

The Examiner further states on page 4 of the Office action that "In other cases, such as in the references above, synergy between components was investigated". Applicants assume that the Examiner is referring to, for example, Borisy *et al.*, which describes effects from random combinations of isolated drugs. However, none of the cited documents suggests identifying **more than one** sub-components of natural products that act synergistically. The present invention, in contrast to the cited references, is based on a holistic approach to develop new medicaments (see, e.g., Specification, page 2, lines 17-30).

Furthermore, the present invention finds bioactive compound profiles that work in synergy using a disease pattern. Other approaches use a single disease marker for a disease evaluation (reductionistic approach), whereas the present invention applies multifactorial disease patterns (systemic or holistic approach).

For the reasons stated above, withdrawal of the Section 103 rejection is requested because the claims would not have been obvious to one of ordinary skill in the art when this invention was made.

Remarks Regarding Section 112 First Paragraph

Claims 1-20 and 25 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the written description requirement because the claims are alleged to contain subject matter which was not described in the Specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the Application was filed, has possession of the claimed invention. Applicants traverse.

The disclosure in the application as filed clearly demonstrates that Applicants were in possession of the claimed invention at the time the application was filed. The Example described on pages 16-18 of the international publication describes a typical experiment of how to implement the claimed method. The Examiner states that the experiment is entirely hypothetical and no subject, disease, biomarker or multivariate analysis is shown. Applicants note, however, that an actual reduction to practice is not a requirement for patentability and is but one of many ways to demonstrate possession of the invention.

The application describes each step of the claimed method in such a fashion that one of skill in the art could practice the invention. The method itself is not limited to a particular subject, disease, or biomarker, but rather, is useful for a number of analyses. Suitable subjects are described on page 6, lines 19-21; suitable diseases are any disease of which a biological profile can be determined (page 7, lines 7-9); suitable biomarkers are described on page 7, lines 19-30.

The Examiner further states that the "specification reveals no natural products, no improvements and no multivariate analysis" (see page 7 of Office Action). The application describes, however, natural products on page 6, lines 14-18 and page 10, lines 27-33. Multivariate analysis is described, for example, on page 14, line 19 to page 15, lines 15. With regard to "improvements", Applicants submit that the application provides a method related to identifying components of natural products having synergistic effects. The Example on pages 18-19 describes how these components are identified by correlating data obtained from, for example, Figures 1, 2, and 3.

In addition, Applicants provide a declaration by Dr. Jan van der Greef showing that one of skill in the art would know how to practice the features of the claims and would be enabled by this Specification to practice, make and use the full scope of the claims.

By using the disclosure of the Specification, Dr. van der Greef performed an experiment to discover synergistic compounds in extracts of cannabis. The objective of the experiment was to find compounds in extracts of cannabis that potentially have a beneficial effect in relation to curing certain diseases. In this case, it is known that inflammation is a core issue in many diseases in living systems, for that reason a cell based inflammation system was used to determine a biological profile of a disease. The biological profile can be determined by comparing the activity and distribution of human monocytes in normal and diseased individual. Methods for such comparison are commonly known. However, correlating experimental results with activities of chemical compounds in a complex mixture have not met with success. It is known that cannabis extract has an effect on inflammatory disorders but the composition of

active ingredients, inactive ingredients and possible harmful ingredients has not been established. One example of an inflammatory disease which we chose for this study is rheumatic disease. See, Dr. van der Greef's declaration, paragraph 8.

The experimental approach involved the following steps: (a) extracting different variations of cannabis using different extraction methods (such as, for example, hot extraction, cold extraction and determination of the composition of extraction liquid) to provide different concentration of components in the extracts; (b) applying the extracts to different assays using different concentrations of components in the extracts; (c) measuring the effect of the extracts on the assayed biological phenomena using metabolomics based on LC-MS in both positive and negative mode; (d) using analytical techniques to determine the composition of the extracts; (e) relating the chemical composition of the extracts to the measured effects in the assays using multivariate regression techniques (PLS); (f) determining, using variable selection, which chemical compounds relate to the measured effects in an assay; (g) chemically identify the compounds of the extracts which have a correlation with the measured effects in the assay; and (h) performing biological interpretation to determine what the underlying mechanism is for the measured effect in the assay. See, Dr. van der Greef's declaration, paragraph 9.

The data analytical technique used in the research is called PLS (partial least squares), which is a multivariate regression technique. This technique is commonly used when interaction effects are to be expected (i.e., synergistic effect in this case; the effects of one compound is dependent upon the presence of other compounds). While many compounds are measured using the LC-MS technique, a second technique called variable selection is added to the model

estimations. This variable selection technique allows for determining which of the variables (chemical compounds) have a significant effect on the measured effect. See, Dr. van der Greef's declaration, paragraph 10.

Experimental details related to the key procedure of identification compounds from cannabis that are related to biological activity were performed are described below for the two metabolomics procedures used; negative and positive ion mode. See, Dr. van der Greef's declaration, paragraph 11.

The procedure for the detection of synergetic active compounds by positive ion mode metabolomics is described in detail below. As described above, LC-MS data of cannabis extracts obtained in the positive mode were correlated to the selected bioassay results using PLS models. From this exercise, several peaks in the LC-MS data significantly correlated with the bioassay results (see Table 1). Of the seven correlating peaks, three could be chemically identified as AcCBD, CBG and CBC. Four other compounds remained unidentified. Using high-resolution MS, we tried to elucidate the elemental composition of these unknown peaks using the exact mass. MS/MS experiments were performed to identify the compounds. Firstly, some of the cannabis extracts, including the new cannabis extract, were analyzed by LC-MS to verify whether the correlating compounds were still present. In every extract, all compounds in Table 1 could be detected. The exact mass of AcCBD, CBG and CBC resulted in an elemental composition that was in agreement with the structural formula of these compounds. For the peak with m/z 377, it was observed that the highest m/z value of this peak was 375.2166 corresponding to $C_{22}H_{31}O_5$. This elemental composition corresponds well with that of either

hydroxy-AcCBD or hydroxyl-THC-acid. Two peaks with the same exact mass are visible in the extract of CBD1-4 and also in the standard solution of cannibinoids CBG, CBD, CBC, CBN, THC, AcCBG, AcCBD and THC-acid. In the standard solution, the two peaks are of equal height while, in the extract, one peak is dominating. Based on the retention time and the relevant peak areas of this peak in the extracts compared to the concentration of cannabinoids in the same extracts, this peak could be identified as hydroxyl-THC-acid. The peak with m/z 203 could not be detected with the FT-MS due to its low abundance. For the peaks with m/z 219 and 262, an elemental composition could be determined to be $C_{15}H_{32}O_1$ and $C_{17}H_{25}O_1$, respectively. Database searching using SciFinder resulted in about 1300 possible structures. Hence, it was not possible to identify these compounds. See, Dr. van der Greef's declaration, paragraph 12.

Table 1: Results of identification of correlating compounds detected in the positive ionization mode

m/z	t_r (min)	Identity	Exact mass	Elemental composition	Remark
377	14.8	OH-THC-acid	377.2322	$C_{22}H_{33}O_5$ $[M+H]^+$	Highest m/z 375.2166 $C_{22}H_{31}O_5$ $[M+H]^+$
219	19.3	?	219.1742	$C_{15}H_{23}O_1$ $[M+H]^+$	
203	21.2	?	-	-	No peak was detected in FT-MS due to low abundance of this peak
359	23.4	AcCBD	359.2216	$C_{22}H_{31}O_4$ $[M+H]^+$	
262	23.7	?	262.2165	$C_{17}H_{28}O_1N_1$ $[M+NH_4]^+$	$C_{17}H_{25}O_1$ $[M+H]^+$
317	24.0	CBG	317.2475	$C_{21}H_{33}O_2$ $[M+H]^+$	
315	29.0	CBC	315.2318	$C_{21}H_{31}O_2$ $[M+H]^+$	

See, Dr. van der Greef's declaration, paragraph 13.

In a parallel procedure, we performed experiments to detect synergistic active compounds by negative ion mode metabolomics. The LC-MS data obtained in the negative ionization mode was also correlated to the selected bioassay results using PLS. Looking only at correlations with weighted importance > 90% resulted in about 32 relevant hits, i.e. mass.retention times. Further investigation of these hits, i.e. deleting noise and combining mass.retention times belonging to the same peak, i.e. compound, resulted in 12 relevant peaks (Table 2). Three peaks were tentatively identified as hydroxyl-cannabinoids based on the elemental composition and retention times. The elemental composition of the remaining three peaks, i.e. 371, 279 and 255, showed similarity with those of cannabinoids. However, database searching using SciFinder resulted in more than 1000 possible structures and therefore these peaks could not be conclusively identified. See, Dr. van der Greef's declaration, paragraph 14.

Table 2: Results of identification of correlating compounds detected in the negative ionization mode

m/z	t_r (min)	Elemental composition $[M-H]^-$	(Tentative) Identity	Remarks
371.1858	18.3	$C_{22}H_{27}O_5$	?	
375.2171	20.1	$C_{22}H_{31}O_5$	hydroxy-AcCBG	
357.2065	22.1	$C_{22}H_{29}O_4$	AcCBD	-
359.2221	22.3	$C_{22}H_{31}O_4$	AcCBG	-
347.2260	22.6	$C_{21}H_{31}O_4$	dihydroxy-CBG	MS/MS shows two times loss of H_2O

357.2066	24.2	$C_{22}H_{29}O_4$	THC-acid	-
331.2277	24.7	$C_{21}H_{31}O_3$	hydroxy-CBG	MS/MS shows two times loss of H_2O
315.2330	25.6	$C_{21}H_{31}O_2$	CBG	Highest m/z corresponds to [M-H+CH3COOH]$^-$
313.2172	25.9	$C_{21}H_{29}O_2$	CBD	Highest m/z corresponds to [M-H+CH3COOH]$^-$
313.2174	29.7	$C_{21}H_{29}O_2$	CBC	Highest m/z corresponds to [M-H+CH3COOH]$^-$
279.2330	31.0	$C_{18}H_{31}O_2$	?	
255.2331	35.7	$C_{16}H_{31}O_2$	?	

See, Dr. van der Greef's declaration, paragraph 15.

The results of this research, as shown above, show that a number of extracts have a significant effect on the bioassay. Specific bioassays which were used to determine these effects is the assessment of inflammation of monocytes including (1) TNF-induced inflammation and (2) LPS-induced inflammation. These inflammations can be monitored directly or via inflammation markers which include, at least, the markers used in this study which include NF-kB; IL1, IL6, IL8, TNF-a, and PGE2. The multivariate technique used to correlate the bioassay data with the metabolomics data (both positive and negative ion mode) belongs to the so-called multivariate regression techniques in particular Partial Linear Squares (PLS). Our results indicate that compounds originating from the cannabis plants and extracted during the extraction procedure have a synergistic effect. Using the novel approach claimed in this application, it is possible to trace and identify those components (i.e., compounds in Tables 1 and 2) among a multitude of other components in the plant extract which have little or no biological effect in our assays. See, Dr. van der Greef's declaration, paragraph 16.

Withdrawal of the written description rejection made under Section 112, first paragraph, is requested because the specification conveys to a person skilled in the art that Applicants were in possession of the claimed invention as of the filing date. Their disclosure would also teach a skilled person, who possesses general knowledge available in the art, how to make and use the claimed invention.

Remarks Regarding Double Patenting Rejection

Claims 1-20, 25 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-16 of copending Application No. 10/571,087. Applicants traverse.

Applicants traverse because after an indication of allowable subject matter, they may submit a terminal disclaimer, cancel conflicting claims 1-16 of copending Application No. 10/571,087, or amend the pending claims or the claims of copending Application No. 10/571,087 with a nonobvious limitation. To require submission of a terminal disclaimer at this time would place an undue burden on Applicants because there is no present guidance on the subject matter that would be allowed in this application or in copending Application No. 10/571,087.

The withdrawal of this provisional double patenting rejection is requested.

CONCLUSION

Having fully responded to the pending Office Action, Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if additional information is required.

Respectfully submitted,

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